

MECHANISMS OF RAPID RECEPTIVE FIELD REORGANIZATION IN RAT
SPINAL CORD

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Dissertation Prepared for the Degree of
DOCTOR OF PHILOSOPHY

UNIVERSITY OF NORTH TEXAS

August 2002

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Vu, Hung, Mechanisms of rapid receptive field reorganization in rat spinal cord.

Doctor of Philosophy (Biology/Neuroscience), August 2002, 51 pp., 3 tables, 10 illustrations, references, 85 titles.

Rapid receptive field (RF) reorganization of somatosensory neurons in the rat dorsal horn was examined using extracellular single unit recording. Subcutaneous injection of lidocaine into RFs of dorsal horn neurons results in expansion of their RFs within minutes. The expanded RFs appear adjacent to or/and proximal to original RFs. Out of 63 neurons tested, 36 (58%) show RF reorganization. The data suggest that dorsal horn of spinal cord is one of the initial sites for RF reorganization. The neural mechanisms of this effect are not well understood. We propose that changes in biophysical properties (membrane conductance, length constant) of the neurons resulting from lidocaine injection contribute to RF reorganization. Iontophoretic application of glutamate onto dorsal horn neurons that show lidocaine induced RF's expansion were used to test the model. Application of glutamate produced reduction of reorganized RFs in 9 of 20 (45%) tested cells. Application of NBQX produced no effect on either original or expanded RFs indicate that RF shrinkage effects of glutamate involve NMDA receptors. The results are consistent with the prediction of the proposed model.

Subcutaneous injection of capsaicin into tactile RFs of low threshold mechanoreceptive dorsal horn neurons produced no effect on the RF sizes that are consistent with other studies. Following the injection, the original RFs were completely silenced (46%) or remained responsive (54%).

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CHAPTER I

INTRODUCTION

The main focus of this study was an examination of the processes that underlie reorganization of tactile peripheral receptive fields (RFs) resulting from lidocaine-induced deafferentation. RF reorganization is defined as a shift in RF position that occurs within minutes following subcutaneous lidocaine injection into a tactile receptive field. This reorganization has been observed throughout the somatosensory system. While it appears that activation of previously ineffective synapses may underlie the change in RF, the specific mechanism underlying this unmasking effect is unknown. The current study tested a new model that could explain the unmasking process. According to this model, a reduction in background synaptic activity changes a neuron's membrane conductance (due to increased channel closure time), and hence its length constant, resistance and electrotonic length; as a result, previously ineffective distal synapses are able to influence membrane potential at the soma. The model was tested in the spinal cord dorsal horn using in vivo extracellular recording methods. In the first part of the study, the characteristics of lidocaine-induced RF reorganization in dorsal horn cells were studied. The effectiveness of capsaicin injections in producing RF reorganization were also studied because C-fiber input has been proposed as the source of masking inhibition in the somatosensory system and subcutaneous capsaicin injection produces RF

reorganization in the dorsal column nuclei. The final part of the study involved the use of iontophoretic application of pharmacological agents to directly open ion channels while monitoring changes in the sizes of reorganized RFs. This approach provided a direct test of the model of RF reorganization described above.

Receptive Field Reorganization in The Central Nervous System

Somatotopic representations in the nervous system can be defined on the basis of body maps, as well as by single neuron receptive fields (RFs; skin areas to which a somatosensory neuron will respond when the appropriate stimulus is applied). Perturbations of the inputs to somatosensory neurons cause changes in these representations, suggesting that the somatosensory system is an adaptive system - capable of undergoing structural and functional changes in response to changes in peripheral sensory input. Changes to body maps typically occur following long-term manipulations such as amputation or training (reviewed by Buonomano et al., 1998; Kaas et al., 1999; Garraghty and Kaas, 1992). Changes in RFs can occur very rapidly (within minutes) following a change in the activity of peripheral afferents (reviewed by Snow and Wilson, 1991; see also Calford and Tweedale, 1988; Pettit and Schwark, 1993). The following review is focused on the process of rapid RF changes.

Rapid RF changes can occur at all levels of the somatosensory system. These changes were first described in thalamic neurons of the cat by Nakahama and colleagues (1966). Injection of procaine into the RFs of these neurons resulted in rapid expansion of the RFs within minutes, such that the neurons began to respond to skin areas to which

they had been unresponsive prior to injection. Since this early study, similar effects have been reported in somatosensory cortex (monkey: Calford and Tweedale, 1991c; cat: Calford and Tweedale, 1991a; Metzler and Marks, 1979; raccoon: Rasmusson and Turnbull, 1983; Kelahan and Doetsch, 1984; Turnbull and Rasmusson, 1990; flying fox: Calford and Tweedale, 1988, 1990, 1991b; rat: Byrne and Calford, 1991; Nicolelis et al., 1993 and Shin et al., 1991), in thalamus (rat: Nicolelis et al., 1993; Alloway and Aaron, 1996; raccoon: Rasmusson et al., 1993; Rasmusson, 1996), in dorsal column nuclei (cat: Pettit and Schwark, 1993; Dostrovsky et al., 1976; rat: Panetsos et al., 1995; Panetsos et al., 1997 and Faggin et al. 1997). In all of these studies, RF changes were noted when afferent input from peripheral receptors was reduced following amputation, anesthesia, or central blockade of afferents.

Following reductions in input, unmasked RFs appear within 2 to 15 minutes and are usually located proximal and adjacent to the original RFs. The sizes of these unmasked RFs vary from one half to four times the size of the original RFs. The spontaneous firing rates of neurons following blockade of their afferent input may increase, decrease or remain unchanged. The unmasked RFs may remain for hours (from 30 minutes to more than 6 hours). Silencing only capsaicin-sensitive afferent fibers is also sufficient to induce RF reorganization (Calford et al., 1991; Pettit and Schwark, 1996). In some cases, the unmasked RFs are not simple expansions of the original field, but also include changes in modality and/or adaptation rate (Nakahama et al., 1966; Pettit and Schwark, 1993; 1996). For this reason, Pettit and Schwark (1993; 1996) termed this effect RF reorganization, rather than expansion. The data from these studies suggest that

RFs are not static, but instead can change size to reflect changes in the level of peripheral activity. This self-adjusting ability of the somatosensory system may reflect a fundamental organization of the central nervous system. When there is a high level of peripheral input activity (such as during tactile scanning of a surface), RF sizes are small and discrimination capability is high. In contrast, in response to a decline in afferent signals, the RFs increase in size and detectability is enhanced.

There is evidence for RF reorganization in humans. After anesthetizing the thumbs or lips of human subjects by injection of lidocaine, Gandevia and coworkers (1999) used psychophysical methods to assess changes in RFs. They discovered that the perceived size of thumbs and lips increased by 60-70%.

Neural Mechanisms Underlying Rapid Receptive Field Reorganization

RF reorganization might result from anatomical changes, such as sprouting and formation of new synaptic connections, or from changes in the efficacy of synapses. Because RF reorganization occurs within minutes following inactivation, sprouting and synaptogenesis are unlikely to be responsible. It has therefore been proposed that the strength of “silent” or ineffective synapses may be increased as a result of the inactivation. There is accumulating evidence for the existence of ineffective synapses in the somatosensory system. Wall (1977) has suggested that a significant number of nerve terminals are normally ineffective in spinal cord, and that they only become effective after the normal functioning of afferent fibers are blocked. He based this suggestion on the observation that the extent of physiological receptive fields in cat dorsal horn is

smaller than the anatomical extent of the primary afferents. This sort of mismatch has also been reported for the cat dorsal column nuclei and somatosensory cortex (Fyffe et al, 1986a,b; Weinberg et al, 1990; Landry and Deschenes, 1981). More direct evidence for the presence of ineffective synapses was obtained during RF mapping in somatosensory cortex paired with antidromic activation of thalamic inputs (Snow et al., 1988).

Thalamocortical neurons could often be antidromically activated from cortical stimulation sites that lay outside the corresponding RF borders. Thus, ineffective synapses may be present throughout the somatosensory system. If these synapses, presumably located on distal dendrites, become strengthened, the RF should expand. This, in turn, would directly affect the sensory capabilities of the system.

A mechanism that has been proposed to underlie the unmasking of ineffective synapses is a decline in tonic inhibition. Inhibition apparently plays an important role in somatosensory processing. Many somatosensory neurons have RFs that are comprised of an excitatory region surrounded by an inhibitory region (Mountcastle and Powell, 1959; Mountcastle, 1984; Laskin and Spencer, 1979). The inhibition probably arises from GABAergic neurons. Immunocytochemical studies have revealed GABAergic neurons at all levels of the somatosensory system including the primary sensory cortex (e.g., Li and Schwark, 1994), thalamus (e.g., Spreafico et al., 1983), DCN (e.g., Heino and Westman, 1991) and dorsal horn (e.g., Todd and McKenzie, 1989; Powell and Todd, 1992). Blockade of GABA_A receptors results in RF expansion in the majority of neurons studied in the cortex (Hicks et al., 1986; Hicks and Dykes, 1983; Alloway et al., 1989) and DCN (Schwark et al., 1996). In the somatosensory cortex of cat and monkey, blockade of

GABAergic transmission induced RF enlargement ranging from 2 to 4 times the size of the original RF (Dykes et al., 1984; Alloway et al., 1989; Alloway et al., 1991). These effects appear to be specific to inhibition, because application of the excitatory neurotransmitter glutamate seldom results in RF expansion, even though spontaneous activity may be increased (Dykes et al. 1984; Alloway et al., 1989; Schwark et al., 1999). In a study of RF reorganization in rat DCN, Panetsos et al. (1997) noted that deafferentation produced by lidocaine injection had different effects, depending on the type of neuron. A majority (though not all) of neurons with low spontaneous firing rates showed increased spontaneous activity, while most neurons with high spontaneous firing rates showed decreased activity. Based on antidromic activation from the medial lemniscus, the authors suggested that low firing rate neurons project to the thalamus, whereas high firing rate neurons are interneurons. From these observations the authors proposed that high firing rate neurons are inhibitory, and that reduced firing in these neurons following lidocaine injection releases low firing rate neurons from inhibition, allowing them to respond to additional inputs and thereby gain new RFs. However, this scheme implies a great deal of specificity in the connections of afferent inputs (i.e., predominantly on inhibitory neurons). Furthermore, the failure of glutamate to affect the RF size implies that RF reorganization is not simply due to increased excitation following removal of tonic inhibition.

In experiments conducted to look for the afferent source underlying tonic inhibition, Calford and Tweedale (1991a) applied capsaicin to a peripheral nerve to block afferent C-fibers and observed rapid RF reorganization in somatosensory cortical

neurons. The capsaicin-induced RF expansions were comparable to those produced by lidocaine injection or amputation, and the authors concluded that afferent C-fiber input could give rise to the tonic inhibition unmasked during RF reorganization. In the ventroposteriolateral thalamus (VPL) of the raccoon, lidocaine and capsaicin injections have somewhat different effects. Capsaicin produces new small excitatory RFs whereas lidocaine produces new small excitatory and large inhibitory RFs (Rasmusson, 1993). From these observations, Rasmusson postulated that lidocaine-induced and capsaicin-induced RF reorganization arise from different mechanisms. Pettit and Schwark (1993) found that all of their recorded neurons in the DCN reorganized after capsaicin injection, and that the reorganization are comparable to those produced by lidocaine.

In the present study, capsaicin was injected subcutaneously into the RFs of dorsal horn neurons to investigate the contribution of C fibers to RF reorganization at this level of the somatosensory system. Such experiments have been done previously, but within the framework of studying the mechanisms of pain perception. Typically, the effects of capsaicin injection were studied on the physiology of wide dynamic range and nociceptive-specific dorsal horn neurons. Such injections tended to enhance sensitivity of these neurons in responding to both noxious and innocuous stimuli (Cook et al., 1987, Dougherty et al., 1992, Lin et al., 1997, Thompson et al., 1993). In the few examples of capsaicin injection into the RFs of low threshold mechanoreceptive neurons, sensitization was not observed but instead these neurons showed a reduction in tactile response following the injection (Dougherty et al., 1999). Therefore, another goal of the present

study was to compare the effectiveness of capsaicin and lidocaine in producing RF reorganization in dorsal horn neurons.

Unmasking of Ineffective Synapses Due to Changes in the Biophysical Characteristics of Neurons: a New Model

In the central nervous system, neurons do not exist in isolation. A typical neocortical pyramidal cell may receive between 5000 to 20,000 synapses from other neurons (reviewed by Koch, 1999). Many of these synapses are spontaneously active as indicated by spontaneous action potential firing rates of up to 10 Hz in the cortex of waking animals (Woody et al., 1984; Bindman et al., 1983). Synaptic activation as well as fluctuation in ion channel opening may contribute to this spontaneous activity. It has become apparent that changes in synaptic input activity can have a substantial impact on the integrative characteristics of neurons (Holmes and Woody, 1989; Rapp et al., 1992). Thus, a change in synaptic activity can affect membrane conductance and resistance, time constant, length constant λ [$\lambda = \sqrt{\text{membrane resistance } (R_m) / \text{internal resistance } (R_i)}$], and electrotonic length L (defined as anatomical length / λ). The number of open ion channels at any time will directly influence membrane conductance, and a decline in synaptic activity will reduce the number of open ion channels, resulting in a decrease in membrane conductance. This decrease would also cause a decline in the neuron's length constant. These biophysical properties can substantially affect the propagation of distal synaptic potentials, and thus the probability of generating action potentials at the soma.

Modeling studies, based on physiological data collected from *in vivo* recordings and subsequent anatomical reconstructions, suggest that the influence of background activity on neuronal membrane properties is significant (Bernander et al. 1991; 1994; Paré et al., 1998; Destexhe and Paré, 1999; Stuart et al., 1998). For example, in a modeled layer 5 cortical pyramidal neuron, an increase in spontaneous synaptic activity from 0 to 4 Hz causes a 4-fold increase in electrotonic length (reflecting a decrease in the length constant; Fig. 1; Bernander et al. 1991). As a result, a synaptic potential that decays by 63% at the soma when there is no spontaneous activity will decay by 98% when there is 4 Hz spontaneous activity. In other words, spontaneous activity will “mask” inputs on distal dendrites. Based on these findings, our lab has proposed a new model of the mechanisms that underlie RF reorganization. A RF reflects the *effective* inputs to the neuron. Ongoing spontaneous synaptic activity will restrict these effective inputs to the soma and proximal dendrites (Figs. 2 and 3). When spontaneous synaptic activity is reduced by lidocaine injection, the biophysical changes described above will increase the effectiveness of distal synapses, which will be seen as RF reorganization.

Note that such biophysical changes could result from changes in inhibitory as well as excitatory synapses. Thus blockade of GABA receptors by application of an antagonist would not only reduce inhibitory input directly, but would also result in a decrease in membrane conductance as a result of closing Cl^- channels. According to the proposed model, this change would contribute to RF expansion. Enlargements of RFs have been

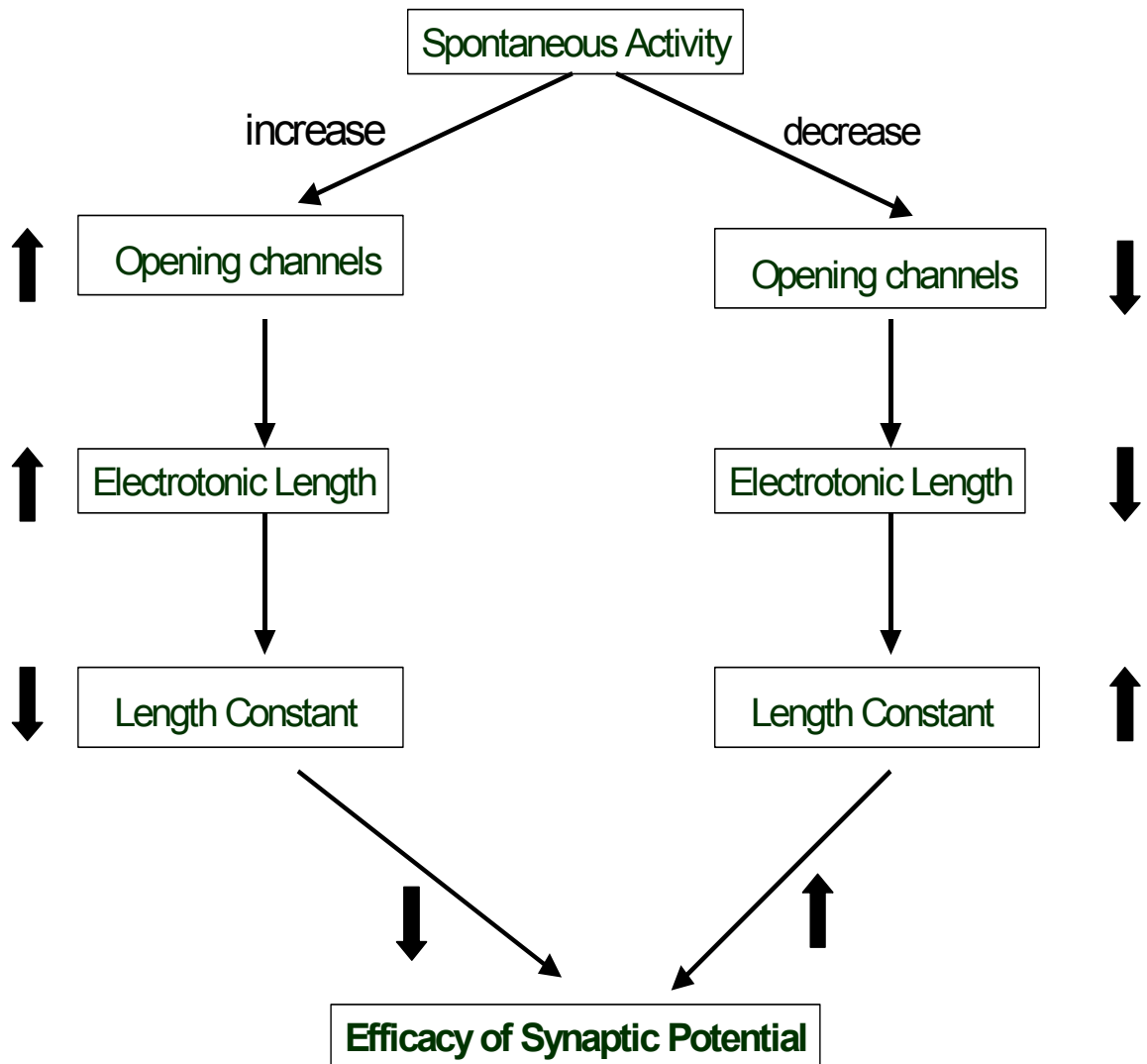


Figure 1. Sequence of effects of changes in spontaneous activity on the biophysical characteristics of a neuron.

observed in the DCN, the thalamus and the somatosensory cortex as a result of GABA_A receptor blockade (Schwark et al., 1999; Hicks et al., 1983, Alloway et al., 1989 and 1991). In contrast, application of glutamate produces little effect on RF size (Schwark et al., 1999; Hicks and Dykes, 1983; Alloway et al., 1989). Because glutamate application opens channels, it might be predicted to cause a decrease in RF size. However, the increased influx of cations mediated by glutamate channels would depolarize the membrane and lower the threshold for generating action potentials. Thus, a lack of effect of glutamate on RF size may be due to counterbalancing effects of increased membrane excitability and reduced length constant.

Receptive field reorganization in dorsal horn neurons

Within the somatosensory system, tactile information is processed in three hierarchical steps: first order neurons in the dorsal root ganglia, which have or contact peripheral tactile receptors, send their axons to the DCN through dorsal columns. Axons of the second order neurons in the DCN then decussate and project as the medial lemniscus to the thalamus. The thalamic neurons then relay information to ipsilateral primary somatosensory cortex. Integration and modification may occur at each of these levels, and as a result of the hierarchical organization changes at lower levels may influence higher centers. Therefore, it is useful to study the mechanisms of RF reorganization at the first site of convergence in order to prevent potentially confounding

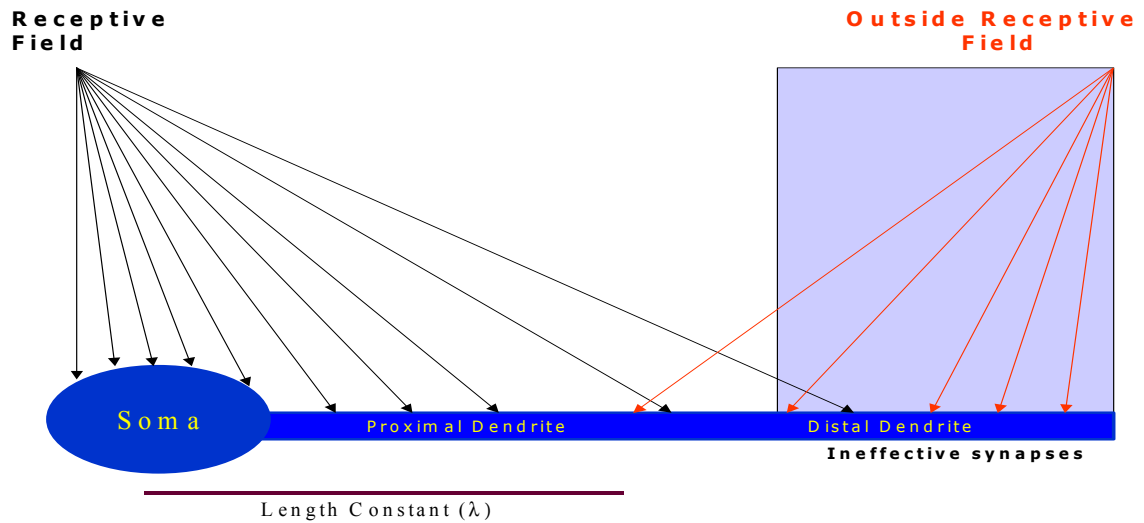


Figure 2. Synaptic terminations that effectively produce the RF are formed on the soma and proximal dendrites, while terminals from outside the RF are formed on distal dendrites. A shorter length constant (λ) causes the distal synapses to be masked.

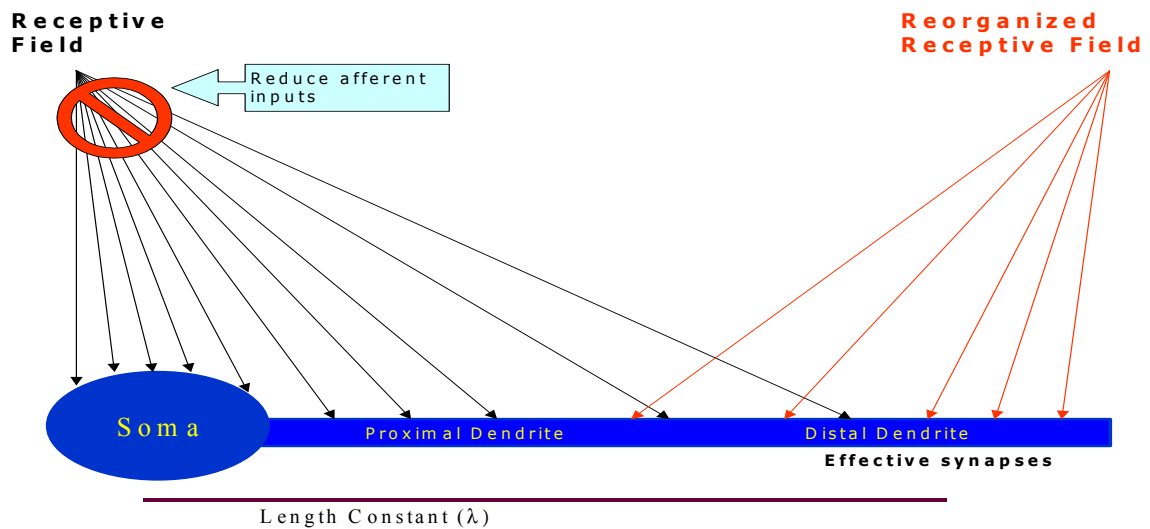


Figure 3. Blocking afferent inputs within the normal RF reduces conductance, which leads to expansion of λ . Thus, distal synapses become effective, as reflected by RF reorganization.

effects. Previous experiments in this laboratory were done on the DCN because these nuclei receive direct input from primary afferents. However, results from another lab suggest that dorsal horn neurons contribute afferents that can mediate RF reorganization in the DCN. When Dykes and Craig (1998) silenced dorsal horn projections to the DCN with lidocaine or cobalt chloride, they observed RF reorganization in DCN neurons. Therefore, if peripheral lidocaine injections produce RF reorganization in the dorsal horn, these changes may contribute to the changes seen in the DCN. There is direct as well as indirect evidence for RF reorganization in the dorsal horn. Lidocaine-induced RF expansion in cat dorsal horn has been reported at a meeting (Stephens et al., 1997). During recordings from rat dorsal horn neurons that receive sciatic nerve input, application of lidocaine to the sciatic nerve or application of strychnine directly onto the neurons reveals new responses to saphenous stimulation (Biella and Sotgiu, 1995). Application of glutamate onto the neurons does not produce the same effect. These results are compatible with the proposed model. In the cat dorsal horn, application of 4-aminopyridine (4-AP), an antagonist of K^+ channels, produces RF expansion (Saadé et al., 1985). Reduction of dorsal horn neuron RFs is seen following iontophoretic application of glycine, a common inhibitory neurotransmitter in mammalian spinal cord that opens Cl^- channels (Zieglgansberger and Herz, 1971). These data suggest that the dorsal horn is also a site of RF reorganization.

In addition to activity-dependent changes in biophysical properties of dorsal horn neurons, other mechanisms may exist in the dorsal horn that control RF size. RF enlargement has been observed following activation of nociceptive inputs to the dorsal

horn (Hoheisel et al., 1993; Hylden et al., 1989; Laird and Cervero, 1989; McMahon and Wall, 1984; Simone et al., 1989; Woolf and King, 1989 and 1990; Millan, 1999). The RF enlargements observed in these experiments required increased activity in nociceptive afferent inputs; in contrast, tactile RF reorganization results from reduced afferent input. Furthermore, these changes in RF sizes predominantly occur in nociceptive specific and wide dynamic range neurons. Sensitization of dorsal horn neurons appears to contribute to these RF reorganizations, and involves the release of substance P, nitric oxide, glutamate and changes in the efficacy of NMDA receptors (reviewed by Millan, 1999). Thus, in the present study, recorded neurons were classified as low threshold mechanoreceptive (LTM), wide dynamic range (WDR) or nociceptive specific (NS) and the characteristics of RF reorganization of each class were examined.

The specific objectives of this study were 1) to determine if dorsal horn neurons are capable of undergoing rapid RF reorganization following subcutaneous lidocaine injections; 2) to test if direct opening of membrane channels by glutamate application results in a reduction in the size of reorganized RFs, as predicted by the model; and 3) to characterize the effects of capsaicin injections on the RFs of low threshold mechanoreceptive neurons in dorsal horn.

CHAPTER II

EXPERIMENTAL METHODS AND PROCEDURES

Subjects

The experiments were performed on young adult Long Evans hooded rats weighing 250 - 400 g. Animals had food and water available *ad libitum* and were housed on a 12:12 light-dark cycle. The rats were anesthetized with urethane (200mg/100g i.p.) and injected with atropine (0.05 mg/kg, i.m.). Supplemental urethane was given as needed.

Surgery

After the dorsal lumbar region along the vertebral columns was shaved, the animal was mounted in a stereotaxic frame. Heart rate and body temperature were monitored and maintained within physiological ranges. After separation of dorsal skin, the superficial and adjacent musculatures at the lumbar region of the vertebral column were dissected to expose the vertebrae. The lumbar enlargement containing the L₄ spinal segment was then exposed by laminectomy. The vertebral column was held rigid by using hemostats to clamp on spinous processes caudal and rostral to the exposed

site. The meninges at the site where the electrode penetrated were cut to allow smooth entry. The exposed spinal cord was covered with 4% agarose to maintain stability for recording and to prevent the spinal cord from drying out.

Extracellular Recording and Iontophoresis Procedures

The methods were based on those of Schwark et al. (1999). Recordings were made with parylene-coated tungsten electrodes (2 megohm; MicroProbe). The recording electrodes were cemented onto a five-barrel iontophoresis assembly, which was made by pulling five-barrel glass micropipette bundles in a pipette puller (PP-3200, Activational Systems Inc). The tip of the recording electrode was positioned 5-10 μm beyond the tip of the barrel assembly to minimize the diffusion of drugs from the recording site. A remotely controlled micromanipulator was used to lower the electrode assembly into the dorsal horn of the spinal cord. Iontophoresis barrels were filled with L-glutamic acid (100 mM, pH 8), or 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium salt (NBQX, 1 mM, pH 8). The fifth barrel was filled with 165 mM NaCl for current balance. To minimize passive diffusion of the drugs, appropriate retaining currents were applied and the smallest effective ejecting currents were used.

Amplified action potentials from single neurons were viewed on a digital oscilloscope and monitored throughout the experiment. The signals were also fed to a datawave analysis system, which enables separation of multiple waveforms. Triggered waveforms on the screen and an audio amplifier were used to enable the experimenter to map RFs

and their boundaries. Triggered waveforms were stored in a computer for additional off-line analysis.

RF Mapping

RFs were mapped with Semmes-Weinstein monofilaments and the boundaries were drawn on a map. The stability of RF borders was monitored by periodic (5-minute intervals) mapping using either Semmes-Weinstein monofilaments or a computer-controlled stimulator. The computer-controlled stimulator was a solenoid-controlled system that delivered air puffs (to activate hair RFs) or moved a 1 mm metal rod (to activate skin RFs). The inter-stimulus interval and duration of the stimuli were regulated by a digital stimulator. Five stimuli, 4-6 seconds apart, were applied to the RF center, a site on the RF boundary, and outside the RF.

Neuron Classification

Handheld stimuli (brush, pressure, pinch) were used to characterize dorsal horn neurons. Each stimulus was applied for 10 seconds. Neurons that were most sensitive to brush stimuli were classified as low threshold mechanoreceptors (LTM). Neurons that responded to all stimuli, but best to pinch were classified as wide dynamic range (WDR). Neurons that responded mainly to a pinch with self-closing smooth forceps (force of 210g/15 mm²) were categorized as nociceptive specific (NS).

RF Reorganization Methods

Before lidocaine inactivation, RF border stability was monitored for at least 15-20 minutes. If the borders were stable over this period, a volume of 1-10 μ l of 2% lidocaine or capsaicin (10% dissolved in 70% ethanol) was subcutaneously injected into the center of the RF to produce temporary denervation of afferent input as outlined by Pettit and Schwark (1993 and 1996). Five minutes after the injection and then at 10 minute intervals, the RF was mapped using Semmes-Weinstein monofilaments to test for RF reorganization. In some experiments, the computer-controlled stimulator was used to obtain a quantitative record of the RF reorganization.

Drug application methods

Changes in spontaneous activity were monitored to evaluate the effectiveness of drugs and iontophoresis barrels. For some cells, RFs were mapped during drug ejection before lidocaine injection to test the drug's effect on normal RF sizes. The ejecting current was then turned off to allow the neurons to recover, and RFs were remapped to ensure that the RF did not change as resulted of drug application. After RF reorganization had occurred, drugs were administered starting with small currents and increasing incrementally while observing changes in spontaneous activity and RF size. Ejection currents were not increased above the levels that elicited RF changes. Appropriate retaining currents were used to prevent drug leakage. During drug ejection, the RF was

mapped using Semmes-Weinstein monofilaments to test for change in RF size. In some cases, the person mapping the RF was blind to the conditions of drug application.

Histology

In some experiments, a small lesion was made at the end of the recording by applying 5 μ A current for 5 seconds in order to mark the recording site. Animals were perfused intracardially with 0.9% normal saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). After the perfusion, the spinal segment containing the recording sites was removed and postfixed for 1 hour in the same fixative and then cryoprotected in 30% sucrose (in 0.1 M phosphate buffer). 40 μ m sections were cut (coronal plane) through the recording site with a freezing sliding microtome and collected in phosphate buffer. Free-floating sections were then mounted on slides and stained with thionin to localize the recording sites.

Data analysis

Spontaneous firing rates were measured at particular periods throughout the experiment, including before lidocaine and capsaicin injections, immediately after injection, after RF reorganization, during drug application, and after recovery of the original RF. Firing rates during stimulus applications were also measured. RF changes resulting from lidocaine and capsaicin injections or drug application were assessed by comparing RF maps made throughout the experiment. Relations between cell types, depth in spinal cord, modality, spontaneous activity, and glutamate effects on expanded

RFs were assessed. In addition, rapid RF reorganizations in the dorsal horn neurons elicited by lidocaine injection were compared with results from injecting capsaicin.

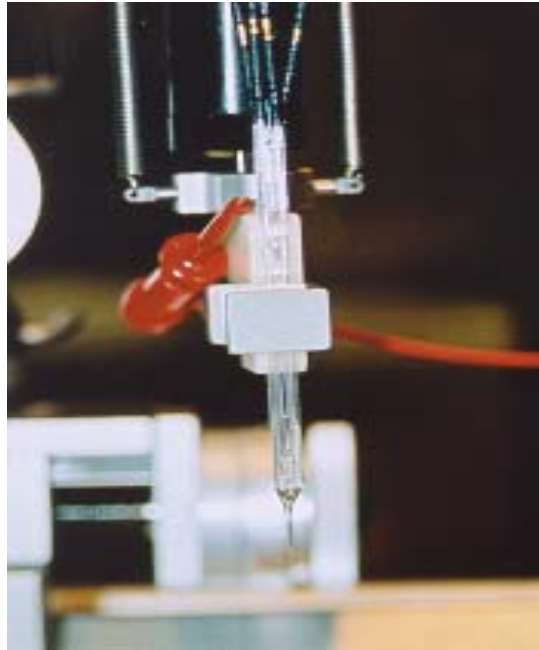


Figure 4. Five-barrel iontophoresis assembly attached to a micromanipulator. A wire was inserted into each barrel passing current to eject drugs at the tip of the electrode.

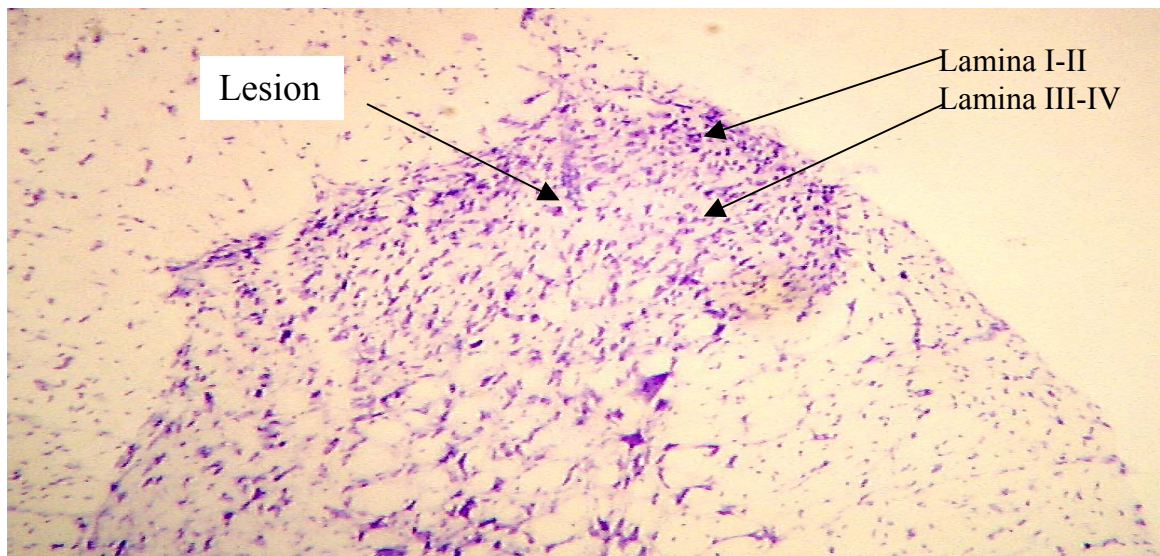


Figure 5. Photograph of Nissl stained lumbar spinal segment contained a recording site. The lesion indicated that the electrode was recording from upper portion of lamina III.

CHAPTER III

RESULTS

A total of 57 animals were used in this study. Seventy-four neurons were recorded: 54 LTM, 1 NS, 9 WDR and 7 non-classified neurons. All of the cells had cutaneous mechanoreceptive fields on the ipsilateral hindlimb or hindpaw. The stability of RF borders was assessed in six LTM neurons: Semmes-Weinstein monofilaments were used for one of the neurons and computer-activated stimuli for the remaining five. The computer-controlled stimuli were applied to the RF center, a site on the RF border, and outside the RF every 5 minutes. The response characteristics at the boundaries and the RF borders remained stable throughout 45-60 minutes of recording (Figs. 6 and 7). The locations of recording sites were verified for 5 recorded dorsal horn neurons using Nissl stain to visualize the lesion. For the remaining 69 neurons, recording sites were estimated from the depth reading of the micromanipulator. The depths of the recording sites ranged from 70 μm to 900 μm , and corresponded to laminae I-VI.

Effects of Lidocaine Injection on Receptive Field Organization

The effects of lidocaine injection on RF organization were tested on 63 dorsal horn neurons. Forty-six of the neurons were low threshold mechanoreceptive neurons (LTM), one was a nociceptive specific neuron (NS), six were wide dynamic range

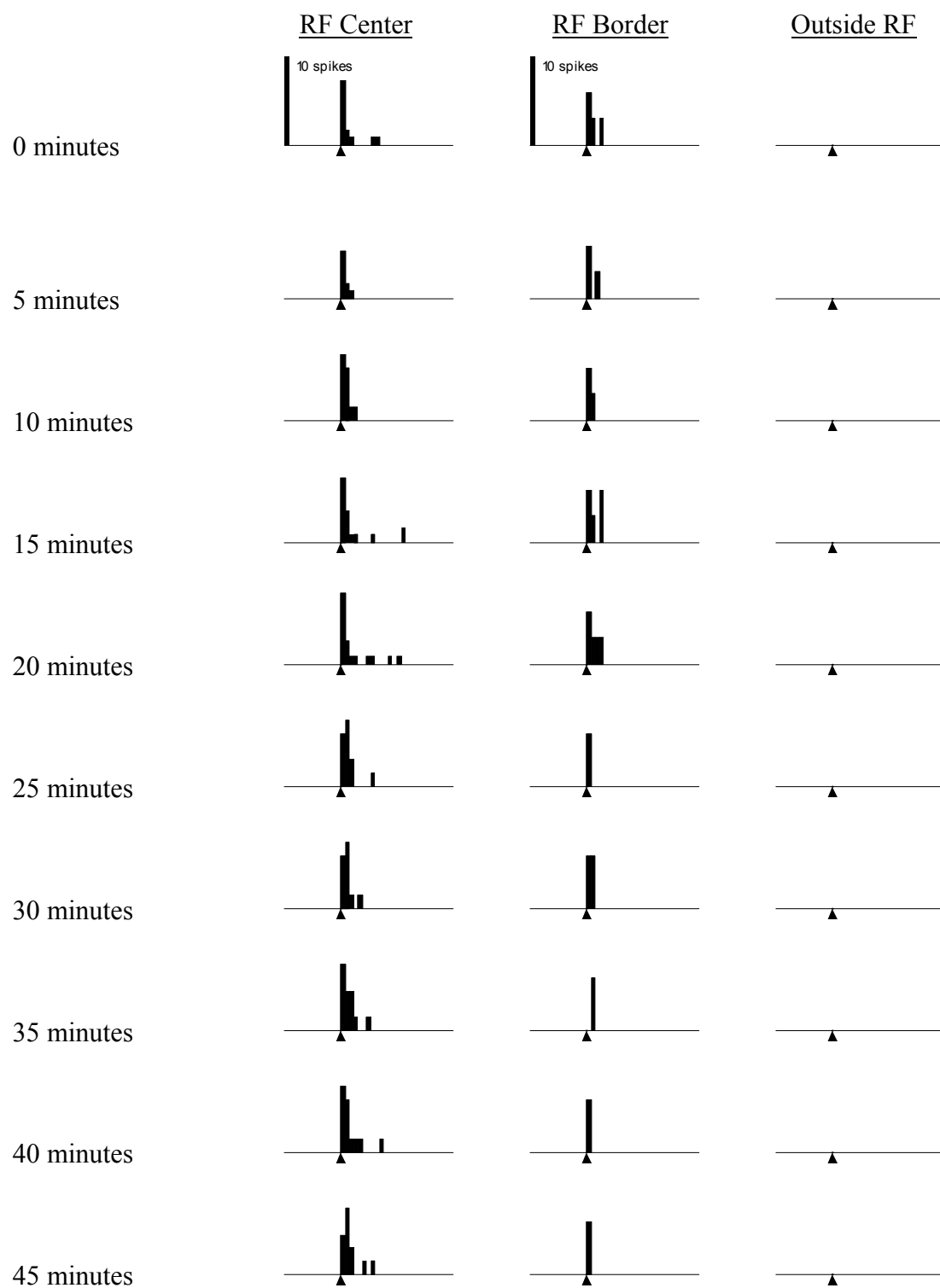


Figure 6. (previous page) Peristimulus time histograms of the responses of a single LTM dorsal horn neuron to 5 stimulus presentations of a pneumatic probe at each of three positions. The stimulus duration was 250 msec. Each peristimulus time histogram is 500 msec long. Arrowheads denote stimulus onset. The neuron did not respond to stimuli applied outside of the RF (position 3). The RF boundaries were stable throughout the 45 min recording period. This neuron did not have spontaneous activity.

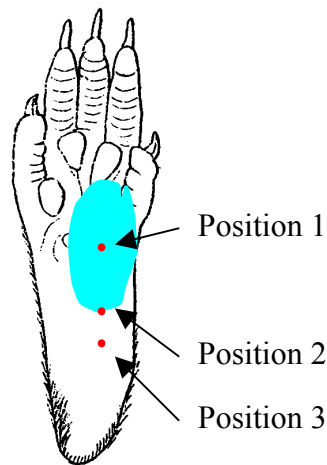


Figure 7. Locations of the three positions on the rat hindpaw where computer-controlled stimuli were applied for Fig.6.

neurons (WDR), and seven were not classified. RF reorganization was observed in thirty-six (58%) of the neurons. Twenty-seven LTM (59%), 1 NS (100%), 5 WDR (83%), and 3 non-classified neurons underwent RF enlargement (Figs. 8 and 9). All reorganized RFs were located adjacent with or proximal to the original RFs. The volumes of lidocaine injected to induce RF reorganization ranged from 3-30 μ l.

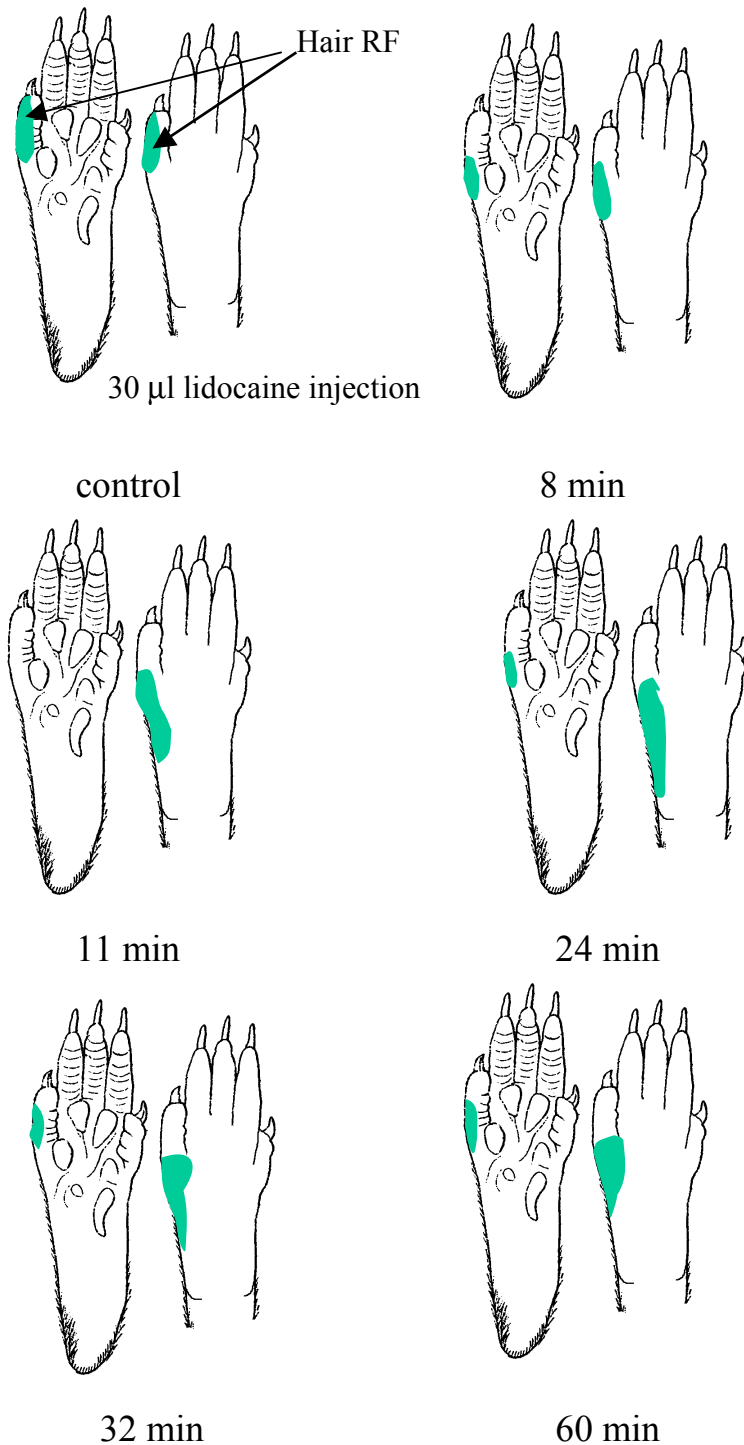


Figure 8. Lidocaine-induced RF reorganization in a LTM dorsal horn neuron. The original RF (control) and reorganized RF were located on hairy skin of the hindpaw. RF expansion occurred 8 min after lidocaine injection. By 60 min the expanded RF had shrunk and a portion of the original RF returned.

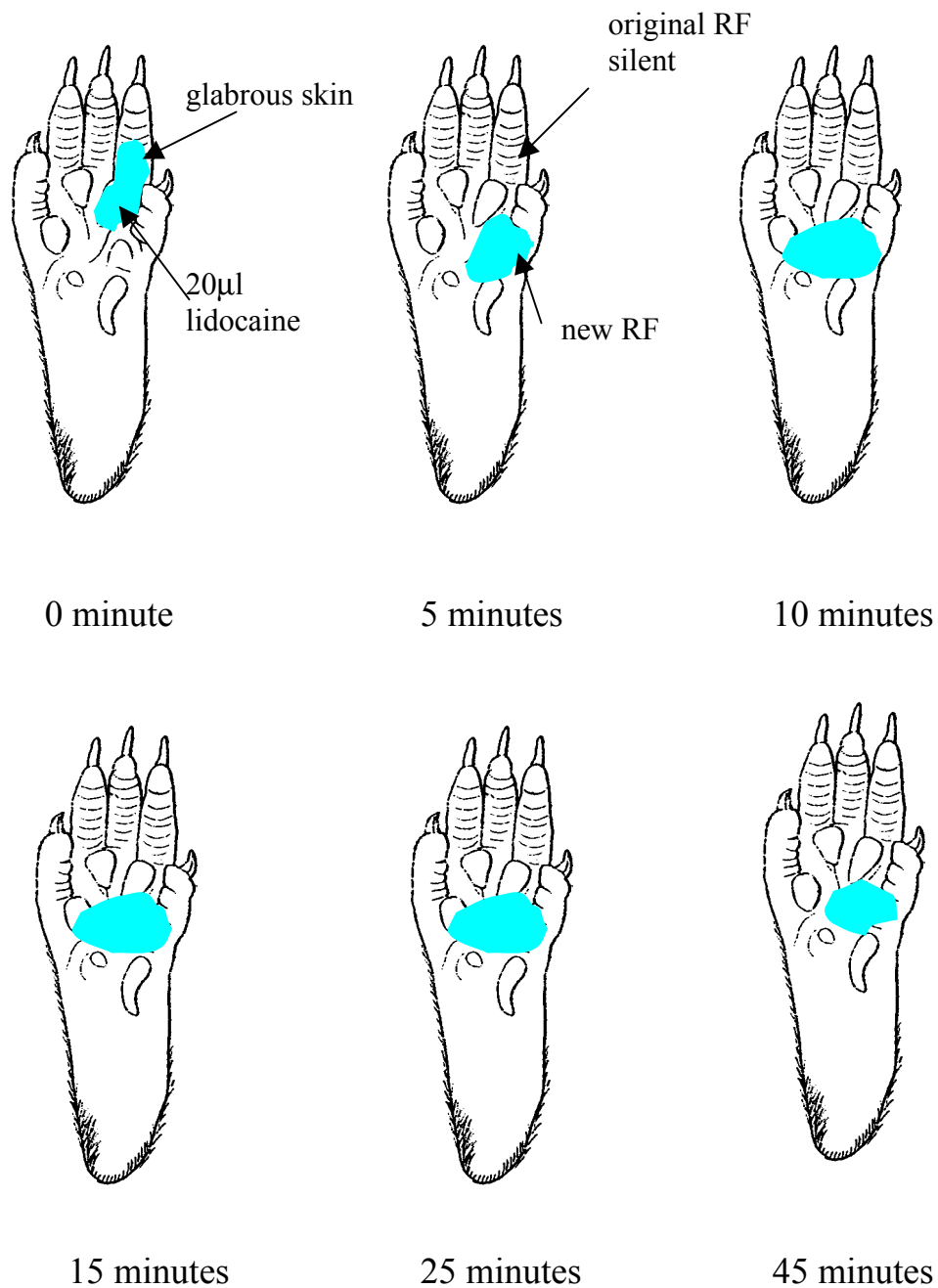


Figure 9. Lidocaine-induced RF reorganization in a WDR dorsal horn neuron. The original RF (0 minute) and reorganized RF were located on the glabrous skin of the hindpaw. Within 5 min after lidocaine injection, RF reorganization occurred and the original RF was completely silenced. 45 min after the injection the expanded RF shrunk slightly while the original RF was still silent.

Reorganized RFs appeared within 5-15 minutes following lidocaine injection. The spontaneous activity of the neurons ranged from 0-10 Hz (mean: 2.24 Hz, median: 0.2 Hz). Only 22% of the recorded neurons had spontaneous activity. And the effects of lidocaine injection on spontaneous firing of these cells were varied (8 increased, 5 decreased and 1 was unchanged). Following lidocaine injection, almost all of the original RFs remained silenced throughout the 45-60 minute recording period regardless of the presence or absence of RF reorganization. The two exceptions were neurons for which a portion of the original RF regained responsiveness. The sizes of the expanded RFs were approximately 45-200% of the original RFs. The reorganized RFs remained stable throughout the recording period (20-50 min). There were no apparent differences between the modalities of the original and reorganized RFs. There were also no apparent relations between the sizes of reorganized RFs, cell location in dorsal horn. Interestingly, it appears that RF reorganization occur more often in the dorsal horn neurons whose RFs include both digits and plantar surface or dorsal surface (Table.1). It is also noted that there is a relation between the volume of injected lidocaine, modality of neurons and the ability to undergo RF reorganization (Tables 2 and 3).

Effects of glutamate and NBQX on RF reorganization

The effects of glutamate were tested on 21 dorsal horn neurons: 16 LTM, 2 WDR and 3 non-classified. All but 3 neurons responded to glutamate with increased spontaneous activity. Twelve neurons were tested with NBQX: 11 LTM, and 1WDR. Application of NBQX produced no effect on either the original RFs or the reorganized

RFs. Iontophoresis of glutamate prior to lidocaine injection produced a qualitative increase in responsiveness, but had no effect on RF size. After RF reorganization, glutamate reduced the size of the reorganized RFs in 9 (6 LTM, 1 WDR, 2 non-classified neurons) of 20 tested cells (45%) (Fig.10). One LTM neuron showed a further expansion of the reorganized RF after glutamate application. The current that resulted in shrinkage of the new RFs ranged from -2.5 nA to -70 nA. For individual neurons, the effectiveness of glutamate in producing changes in reorganized RFs was not related to the ejection current.

Effects of capsaicin injection on tactile receptive fields

The effects of capsaicin were tested on 11 dorsal horn neurons: 8 LTM and 3 WDR. The volumes of capsaicin injected were 1-5 μ l. Capsaicin affected RF organization in only 2 of the 11 neurons (1 LTM and 1 WDR). In these neurons the newly responsive RFs locate at the areas that are completely separated from original RFs, in contrast to observations of lidocaine induced RF reorganization in which all reorganized RFs appear adjacent to or proximal to original RFs. In six of neurons tested with capsaicin, a portion of the original RF remained responsive to Von Frey stimuli following the capsaicin injection. In the five other neurons the RFs were completely silenced. In two neurons, the RF fell silent within approximately 15 seconds following the injections.

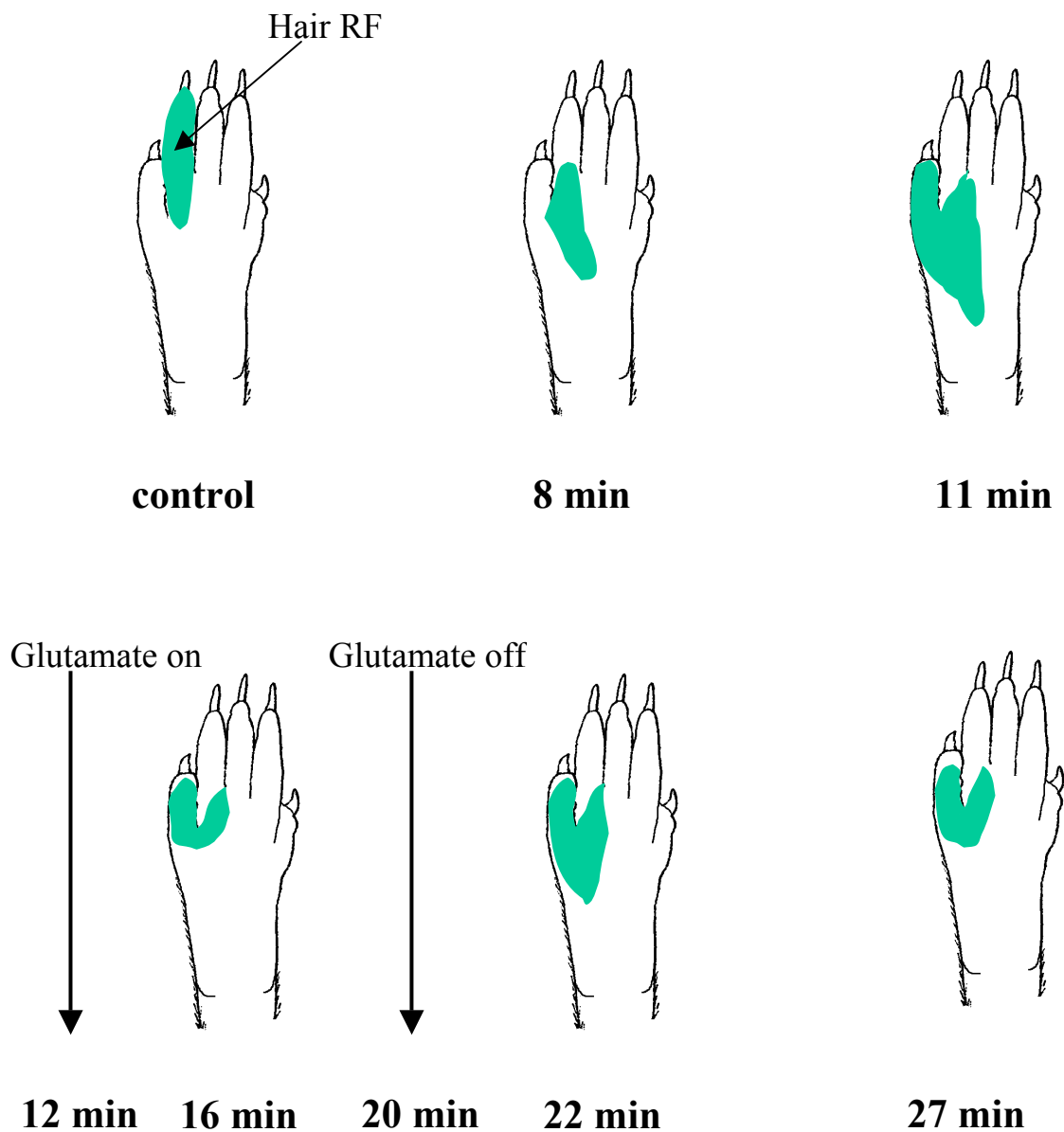


Figure 10. The effects of iontophoretic application of glutamate on lidocaine-induced RF reorganization in a dorsal horn neuron. The RF reorganized 8 minutes after lidocaine injection. Partial shrinkage of the expanded RF occurred 4 minutes after glutamate iontophoresis (-15 nA and -25 nA). Two minutes after terminating glutamate application, the expanded portion of the RF reappeared.

Location of original RFs	Number of cells	Reorganized	Non-reorganized
Digit and plantar or dorsal surface	14	11 (79%)	3
Digit	19	10 (53%)	9
Plantar or dorsal surface	21	10 (48%)	11
Hindlimb	8	4 (50%)	4
Tail	1	1 (100%)	0

Table 1. Distribution of recorded cells according to the location of their cutaneous receptive fields and their response to lidocaine injection.

Modality	Number of cells	Reorganized	Non-reorganized
Glabrous skin	38	20 (53%)	18
Hairy skin	14	10 (71%)	4
Hair	11	6 (55%)	5

Table 2. Responses of different classes of dorsal horn neurons to lidocaine-induced RF reorganization.

Lidocaine volume	Small RF	Medium RF	Large RF
3-5 μ l	7/15 (27%)	6/12 (50%)	0/0
10-20 μ l	4/4 (100%)	8/12 (67%)	4/10 (40%)
25-30 μ l	0/0	3/4 (75%)	4/6 (67%)

Table 3. Proportions of dorsal horn neurons with reorganized RFs by volume of lidocaine injected, categorized by RF size. RF sizes are classified as small (less than 20 mm²), medium (between 20-80 mm²) or large (greater than 80 mm²).

CHAPTER IV

DISCUSSION

The data from the present study demonstrate that denervation of tactile afferent inputs by lidocaine injection affects the RF size of dorsal horn neurons. The results suggest that the dorsal horn of spinal cord is one of the initial site for RF reorganization in the somatosensory system. Almost all of the observed reorganized RFs appeared within minutes after the original RFs were silenced and all were excitatory fields. These results are similar to those found by Pettit and Schwark (1993) in cat DCN. However, there are some differences. In their study, some of the reorganized RFs were inhibitory and all tested neurons underwent RF reorganization; in contrast, in our study of the rat dorsal horn, only 58% of lidocaine injected neurons showed RF enlargement, and all of the expanded fields were excitatory. Functional and species differences, as well as potentially a smaller degree of convergence of somatosensory afferent inputs onto dorsal horn neurons may contribute to these differences. No other study has examined changes in RF size of rat dorsal horn neurons following lidocaine deafferentation; however, Panetsos et al. (1997) used the same method to investigate similar phenomena in rat DCN. He observed that only a small number of cells have high spontaneous activity. They also reported that lidocaine injection had different effects on the spontaneous activity of DCN neurons, and that these differences correlated to some degree with

their levels of spontaneous activity. Results from the present suggest that dorsal horn neurons respond differently. We found no evidence for an effect of peripheral inactivation on spontaneous firing rate of dorsal horn neurons. However, there was a tendency for RF reorganizations occur more frequently in neurons with original RFs that included both the digit and plantar surfaces (or dorsal surface) of the hindpaw. This finding suggests that dorsal horn neuron receiving more convergent inputs may more readily undergo RF reorganization. This suggestion is further supported by the observation that the incidence of RF expansion increases with the larger volumes of injected lidocaine. Similar to the findings of the majority of studies of receptive field reorganization, new RFs were never observed distal to original RFs. It seems likely that fibers carrying information to distal RF locations may run through the injection site, and may thus be also blocked by lidocaine.

The effects of capsaicin injection on RF reorganization observed in the present study are different from those reported at higher levels of the somatosensory system (cortex: Calford and Tweedale, 1991a; Rasmusson, 1993; Pettit and Schwark, 1996) where a majority of tested neurons showed RF reorganization following subcutaneous capsaicin injection. In the current study capsaicin injection produced RF reorganization in only two of the tested neurons. However, these two new RFs were not contiguous with the original RF. Also, in other tests of capsaicin we noted subtle changes in action potential shape that suggested the appearance of a new neuron in the recording. As a result, we cannot be certain that RF reorganized occurred in these two neurons. Interestingly, unlike with lidocaine the original RFs become completely silent within 15

seconds after capsaicin inactivation. This was observed in experiments in which the original RF was stimulated with Semmes Weinstein monofilaments immediately after injection. In several cases, the response from the injected RF became silent during this testing. These findings are consistent with the observation that when the chemical irritant mustard oil is applied to RFs of rat cutaneous mechanoreceptive dorsal horn neurons, none of these cells show any alteration in RF size (Woolf and King, 1990). Moreover, substantial reductions in tactile responses of monkey spinomesencephalic tract cells following intradermal injection of capsaicin have been reported (Dougherty et al., 1999). Both of these observations, together with our findings, suggest that although capsaicin injections produce RF reorganization at higher levels of the somatosensory system, they are ineffective at the level of the spinal cord. It is interesting to note that in the experiments of Dykes and Craig (1998), blockade of spinal inputs to the DCN was sufficient to elicit RF reorganization in the DCN. Perhaps the mechanoreceptive neurons in the dorsal horn that are silenced by subcutaneous injection of capsaicin observed in the present study are responsible for RF reorganization seen at higher level of the somatosensory system following capsaicin injection. This idea raises the question of the role of mechanoreceptive neurons in the dorsal horn during painful stimulation. Dougherty (1999) has observed the capsaicin injections produce sensitization of WDR and NS neurons but that, as in the present study, mechanoreceptive neurons reduce their responses following capsaicin injection. Such a reciprocal response appears to be consistent with the gate control theory of pain formulated by Melzack and Wall (1965).

The data from our drug application experiments suggest that reopening ion channels can reduce the size of reorganized RFs. These results are compatible with our proposed model stating that changes in membrane conductance dictate the effectiveness of distal synaptic potentials at the soma. Thus, changes in background activity may modulate the biophysical properties of a neuron. The failure of NBQX to alter either original or reorganized RF sizes in dorsal horn neurons may indicate that the observed effects of glutamate involve NMDA receptors rather than AMPA and kainate receptors.

It seems likely that changes in membrane biophysical properties are not solely responsible for rapid RF reorganization. Perhaps both mechanisms that have been proposed, tonic inhibition and changes in biophysical characteristics, operate in the somatosensory system to enable this system to adapt to changes in peripheral inputs. Future intracellular studies to directly monitor changes in input resistance and postsynaptic potentials during RF reorganization may clarify the co-existence of these two mechanisms. Other experiments in which the effects of capsaicin injection are studied on antidromically-identified dorsal horn neurons might help to clarify the role of cutaneous mechanoreceptive neurons in acute pain.

In summary, the present data suggest that rapid RF reorganization in rat dorsal horn neurons is produced following lidocaine induced peripheral afferent denervation, and that iontophoretic application of glutamate can reverse this effect. These findings are compatible with the proposed model. Capsaicin injection silenced tactile mechanoreceptive neurons - a result that is consistent with other studies.

Finally, it appears that the dorsal horn of spinal cord is an initial site of RF reorganization. Changes at this level may be reflected in changes at the higher levels in somatosensory system, and must be considered in any interpretation of such changes.

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